Workshop

"Progressing the COREMI working groups objectives"

Montpellier, October 22nd-23rd, 2015

Context, objectives and design

In order to allow the 4 working groups (see below) in the COREMI COST action FA1404 to progress, the workshop 2 in 2015 was held in Montpellier (France). The workshop was dedicated to progress the 2015 objectives (see below), finalise corresponding deliverables and discuss about perspective for future works.

It was designed in such a way as to alternate parallel sessions dedicated to concretely work in small groups and plenary sessions for results presentation and discussions. A plenary introduction allowed to provide participants with a series of information on COREMI and on the Montpellier workshop design (TO BE COMPLETED). Each WG leader presented the global aims and the workshop topic and objectives. After this plenary session, during the first day and the morning of the second day, working sessions were carried out in more or less small groups. Two 3-hour parallel working sessions were assigned to each WG, involving 8-12 main participants + a few "external" participants (coming from other WGs not running at the same time), as well as a 1-hour assessment and brainstorm session per WG, involving the WG leader and any interested people. The second day's afternoon was dedicated to plenary sessions, involving summaries of work by leaders and evaluation by participants, as well as information on the future conference in Croatia in 2016 by Danijela Tomic Horvatec.

	TOPIC	GLOBAL AIM FOR 2015
WG1	Developing alternative control measures	Evaluation of current control methods and strategies for integrating existing and new methods for achieving better red mite control.
WG2	End users (One Health)-interdisciplinary approach	Towards a feasible on-farm monitoring protocol

WG3	Genetic structure in a changing world	Laying the groundwork for molecular studies with a focus on resistances against pesticides
WG4	Epidemiology, pathology, geographical mapping and surveillance tools	Prevalence and effects of PRM infestation: state of art

WG1

Session 1 - 22nd October 9:40-12:40 - ***Attendees: WG1 (novel control methods), plus several members of WG3 (genetics in a changing world)***

The feasibility and creation of a database of European egg producers for the distribution of a Europe-wide questionnaire.

a. Agreed that it was not feasible to create this database prior to the distribution of the questionnaire due mainly to confidentiality issues.
b. A database could be created as from the contact details provided on questionnaire, only by permission of the producer.
c. Data protection of the database must be ensured.

Strategy for the distribution of a Europe-wide questionnaire to egg producers.

d. An introductory talk was given by Rick Van Emous on his experience of carrying out a Dutch survey highlight successes and difficulties.
e. Agreed points on survey strategy were:
    i. Go large – target as many farmers as possible to get an overview of Europe.
    ii. Appoint a country coordinator in each participating country to oversee distribution and collection of questionnaires.
    iii. Use farming press and producer's associations to distribute the questionnaire – the country coordinator to decide best strategy for each country.
    iv. In country coordinator to provide translations.
    v. Provide a 'preferred' online questionnaire option for farmers with a paper back up.
    vi. KISS – keep it short and simple
    vii. Allow anonymity but request contact details and permission to follow up.
    viii. In parallel, provide red mite background and information to the producers on what the survey hopes to achieve e.g. data for umbrella group to lobby legislators, scientific knowledge, inform producers, and identify best control methods and housing practices.
    ix. Include a short section on perceived acaricide resistance.
    x. Follow up and any mite collection to be handled by in-country coordinators.
    xi. Approach the International Egg Commission (IEC) to request funding to host a questionnaire workshop to carry out questionnaire analysis alongside early careers training.

2. **In parallel with the questionnaire - discussion about the feasibility of setting up a 'bio bank' of mite DNA as a generic resource for researchers involved in e.g. phylogenetics or genetics of resistance? If we agree; is a feasible aim for years 2/3:**

a. Agreed it was a sensible idea but only could be achieved as part of the follow up of the questionnaire.
b. Follow up and mite collections to be carried out by in-country coordinators.
c. A greed that a central biobank resource would be useful; details need to be worked out in the future.

Resistance in the field

How to minimise the development of resistance and what advice should we be issuing to producers to prolong the useful life of existing acaricides?

d. Introductory talk provided by Lise Roy covering the concepts of pesticide resistance.
e. Agreed that there was scope for feeding information back to producers including information on: questionnaire results, information factsheets and control guidelines.
f. Producer feedback and red mite information resource should be considered for inclusion into the year 2 and 3 deliverable of the several COREMI working groups.

Session 2 –23rd October 8:30-12:30 - *Attendees WG1 plus several members of WG2 (One health/monitoring) and 4 (epidemiology, pathology and geographic mapping)*

First outline of the European–wide questionnaire

Production of a 1st outline of the European–wide questionnaire for egg producers to evaluate successes and failures of control methods and poultry premises design:

a. The questionnaire should be answered for ONLY ONE building on any given farm, the building containing the oldest flock of 1000 or more birds should be selected.
b. Use Rick Van Emous/Monique Mul's Dutch questionnaire as basis. The following sections and questions were agreed for inclusion of the 1st draft:
    i. Geographical location (no address detail at this point), country and county/province.
    ii. System type – provide tick boxes, care should be taken to explain to in-country coordinators what exactly the system names given describe as there are country to country variation in system design and names used.
    iii. Do you have red mite? Yes/No – but still answer all questions (because this relies on perception rather than fact)
    iv. Include supporting question for red mite presence to gain a view of PRM presence and effect e.g. visible in cracks and crevices, bird mortality etc.
    v. Did you have red mite in this building during the previous flock?
    vi. Treatments, please list ALL treatments, dose used, age of the flock routine i.e. weekly and whole or part of building treated. Give examples of treatments, e.g. chemical names and brand names (include some grey listed ones), water, detergents and naturals and silica.
    vii. Biosecurity questions, e.g. how many shed onsite, egg tray disinfection etc.
    viii. Must include a cost/benefit component to assess perceived economic benefits of the different control options and the thresholds of mite infestation when the farmer believes

control implementation to be of benefit. Suggestion of labour control costs expressed as the number of hours worked on control.

ix. As discussed previously with WG3 on day, inclusion of a section on perceived resistance along with mite sampling for a biobank.

x. Section on human health effects – carefully worded

c. The content and the order and grouping of questions must be compatible with the downstream analysis.

d. Final responsibility for translation into several languages will rest with in country coordinators

e. Several WG1 members have expressed an interest in taking an active role in the questionnaire. A draft questionnaire will be prepared by WG1 lead and distributed to the WG1 questionnaire interest group will for refinement.

Discussion of the integrated control

Discussion of the integrated control, including the refinement of the control compatibility matrix from a review paper currently in draft form (authored by several COREMI members and will acknowledge COST support, it will be listed as a deliverable from WG1):

f. Additional controls not listed in the compatibility table include:

i. Dry ice hygiene treatment (F. Mozafar)

ii. Ozone cleaning (M. Mul)

iii. Emptying shed of equipment and climate exposure (J. Chirico)

iv. Endosymbionts (A.Camarda).

v. trap baiting with bacteria, 2000 publication(J. Chirico)

vi. Trap and biocide, 'miltamycin' (N. Sleeckx)

vii. Duck repellent allomone nutritional supplement, NoReds (M Mul)

viii. Zoomite, no further information (K Bartley)

ix. Intermittent lighting used in Arab states successfully (F Mozafar)

x. Lighting reduce to minimum dark hours (K Bartley)

xi. Electronic perches (Q perch, Vencomatic) (F Mozafar)

xii. Fungi (heat treated spores can enhance virulence) (J Chirico)

xiii. Manure removal

xiv. Commonly used Illegal treatment

g. Additional synergies identified:

i. Pesticide treatment, delay period then apply predatory mites (T. Tuovinen)

ii. Duck allomone and silica is combined in the nutritional treatment NoReds(K Bartley).

iii. Biocide and trap

iv. Silica and natural products (O Sparagano – published by Steenberg)

h. Additional contradictions of integrating treatments:

i. Manure removal and the Duck allomone.

ii. Silica dusts are now banned in Holland (E. Thomas)

iii. 'Spirit' (some disagreement - is this methylated spirit or detergent?) and predatory mites (M Mul)

i. Other points:

i. Dual treatment reduces risk of resistance developing

ii. Some 'natural' treatment are poisonous to hens, e.g. Penny Royal (O Sparagano)

iii. Inclusion of illegal treatments in the review must be carefully considered and the impact assessed.

Action points

1. Appoint an in-country coordinator for each participating country; aim to include as many countries as possible.
2. Contact IEC to ask for funding for an early careers training to analyses questionnaire results.
3. Include producer feedback and information drive as part of year 2 and 3 deliverables.
4. Produce a draft questionnaire (K. Bartley) and circulated for refinement.
5. Liaise with L. Roy and A. Camada on WG overlapping questionnaire content
6. Complete the review of control compatibility with co-authors.
7. Produce an address book of COREMI members and provide COREMI members access. Add skills keywords.

WG2

This summary describes the results and shortly some points of discussion of the two session of Workshop 2 in Montpellier facilitating the goals of Working Group 2 “One Health”.

The session content were focused on the set-up of a monitoring protocol for on farm monitoring of *D. gallinae*. This protocol describes:

1. The monitoring method
2. The frequency and duration of monitoring
3. The places and the number of monitoring places

Collection of monitoring methods

Remarks about specificity of the above mentioned monitoring tools:
Other species were found in some monitoring tools:
other mites than D. gallinae (e.g. predatory mites) (Velcro, early detection method).

Monitoring method or principle	**Reference**
1. ADAS© Mite Monitor	Anonymous 2014
2. Perch trap	Kirkwood 1963
3. Tube containing a fabric or cloth	Maurer et al. 1993
4. Corrugated cardboard/plastic trap	Nordenfors et al. 1999
5. A tube trap with a wooden stick (Rick Stick) or corrugated cardboard (Avivet trap)	Van Emous and Ten Napel 2007 Bronneberg
6. Method for detecting *D. gallinae* in dust, feathers and impurities (early detection method)	Pavlicevic et al. 2007
7. Examining dried droppings for presence of *D. gallinae*	Zenner et al. 2009
8. Mite Monitoring Score (MMS) method	Cox et al. 2009
9. Automated mite counter	Mul et al., 2015
10. Modified trap after Safrit and Arends	Schulz, 2014
11. MTT-Velcro band mite trap	Tuovinen T. (2012-2015) (Luke, Finland)
12. Semi Attractive Trap (SAT)	Chiron et al. 2014 WPSA Stavanger (some French publications, English publication in prep.)
13. Simplified Passive Trap (SPT)	Chiron et al. 2014 WVPA Stavanger (id.)
14. Paper test	Unknown
15. PVC pipe with 13 holes and towel sheet inside	Tucci, Bruno, Tucci, 1988
16. Scout box app	Cropwatch BV
17. Folded paper	Zenner et al., 2009
18. Q-perch counter	Vencomatic
19. Dog (under construction?)	
20. Lohmann trap	Lohmann TZ

No other species found in automated mite counter

Requirements of the monitoring tool for *D. gallinae*

A monitoring tool should be able to:

- Monitor population dynamics
- Monitor spatial distribution
- Detect low numbers of *D. gallinae*
- Determine the effect of interventions
- Provide knowledge about the population on-farm
- Define or determine a threshold

To determine the most favourite monitoring tool, the tools should be checked on:

- Costs
- Durability
- Reliability (which includes: scoring, repeatability, sensitivity, specificity)
- Low handling costs
- Easily implemented in daily management

The two different groups (morning and afternoon session) had different goals:

Group 1. Monitoring should work on-farm and under experimental setting. Monitoring should be done by the farmer. A farm with enriched cages are taken as an example. Automation if available is an advantage. Monitoring during the empty period should not be forgotten.

Group 2. A farmer should be able to monitor the *D. gallinae* population. Monitoring should be executed on layer farms and on rearing farms.

The most favourite method of *group 1* was:
1. Velcro trap
2. Automated mite counter
3. Transparent tube trap

The most favourite of *group 2* was for Layer farms:

- Velcro trap
- Rick stick

The Velcro trap and placing sentinel birds in an empty laying hen house, possibly provides information about the presence of *D. gallinae*.

The most favourite of *group 2* was for rearing farms:

- Velcro trap
- Semi attractive trap
- Rick stick

A suggestion was to develop a new monitoring tool for rearing farms to identify presence of mites, quickly (blood tests?)

Overall the Velcro trap is the most favourite trap of the attendees under all circumstances. It was suggested to give more insight in the method (there is a difference between Velcro with hooks and loops) and the method quality compared with other monitoring methods. Some improvements were suggested; 1) improve the method so the trap could be in the farm for the duration of one week (for practical implementation reasons), 2) develop a scale method for farmers to easily identify the population dynamics, 3) develop a method for automating the counting of the Velcro trap.

Frequency and duration of monitoring

During a sticker session the attendees pointed out the most favourite **frequency** of monitoring (meaning monitoring should be repeated or carried out every day, week, two weeks, month, half a year or once a year) and the **duration** of the monitoring (meaning the time length the monitor should stay in place before it is removed or replaced)

Group 1. suggested for monitoring on farm a two weekly interval and in experimental setting a weekly interval. The duration should depend on the method. Each method has its preference for the duration of the monitoring.

Group 2. advised for practical reasons and for good implementation in the daily management to monitor every week or every two weeks. The duration of the monitoring should be as long as the frequency; one or two weeks.

Overall, the attendees agreed on monitoring with a weekly or a two weekly interval and for a duration of respectively one or two weeks. However, the monitoring method prerequisite the duration of monitoring.

Places to monitor and number of monitoring places

The attendees of the two groups decided upon the places where to monitor and the number of places to monitor the *D. gallinae* population. As suggested by Olivier Bruno this depends upon many different parameters. A good “sampling plan” should be developed with the current available knowledge.

During the session of *Group 1* it was quickly decided to monitor on a cage farm on all sites of each row except on the site near the air inlet, but further evenly distributed with three or four alternating monitoring places on each site of a row. Monitoring should at least be executed at the highest cage level and at the middle cage level. Preferably also monitoring at the lowest cage level should be executed.

In a layer facility with an aviary system the monitors should be placed on the top perches of all rows. Preferably, also monitoring at the lowest cage level should be executed.

Group 2 focused more on the feasibility of the monitoring by a farmer in an aviary system. Some advised to work in alley’s and hang at easy reachable places at 3 to 4 per site of the row. The monitors were placed in alternating way. Others suggested to place them at a reachable high level

and a lower level. This group did not mention to avoid monitoring places at the sites of the rows near the air inlet.

Suggestions for future research

- Comparison of the methods. Even though the most favourite method was picked, the attendees were unable to compare the method as most of the methods were not validated or compared with other methods.
- New monitoring tool for rearing farms to identify the first mite / presence of mites (PCR/ serological tests, other)
- Monitoring methods to be developed for during the empty period (e.g. sentinel birds, attractive traps)
- Finding attractants for *D. gallinae* to find the first mite and improve monitoring
- Set up of a sampling plan for monitoring *D. gallinae* in different housing systems
- Set up of monitoring protocols made for different situations? (e.g. on farm, experimental, aviary, enriched cages,...)
- Develop methods for automatically counting the traps with plastic/ corrugated cards board/ Velcro trap/ ..
- Easy identification method for detecting *D. gallinae* and distinguish it from other mites.

WG3

The 3 main objectives for 2015 in WG3, were as follows:

> Objective 1: forging links to facilitate sample exchange and make molecular explorations in European farms the easiest.
>
> Objective 2: reviewing progresses on genetic markers.
>
> Objective 3: reviewing progresses on 'resistance breaking' from other sectors (e.g. in ticks, *Varroa*, mosquitoes).

In order to answer these, 3 google drive collaborative works were initiated before the workshop and obtained data handled during it, and 3 topics were discussed following an introductory talk.

Google drive collaborative work (first part of session 1)

Three 2-4 persons groups first finalized 3 folders arising from a collaborative work initiated before the workshop using google drive.

1) A tentative survey on skills and facilities for molecular biology, as well as biorepositories (lab, farms) currently available in the COREMI consortium
2) A list of literature references dealing with molecular studies on PRM (.xls format) associated with pdf files (folder)
3) A list of literature references dealing with the genetic bases of insect-/acaricide resistance among arthropods (.xls format) associated with pdf files (folder)

First outline of a survey on molecular facilities and skills in COREMI (collaborative work #1)

Seventeen questions were inserted into the form. Fifteen answers were obtain. Obtained answers were screened in order to get a view of the molecular biology landscape in COREMI. The analysis revealed that the above questions were not clear enough and that the form should be improved in order to get appropriate information. All the more that general discussions between all workshop participants revealed converging need concerning biorepositiories: it was suggested that some work to construct a biobank should be useful to WG3, but also WG1 and WG4. As a result, we consider that first planned deliverable were not possible to be obtained in 2015, but that this first survey would represent a valuable basis for future more collaborative investigations. A summary of obtained information and suggestions for improvement is available in Appendix 1.

Literature reference databases (collaborative works #2 + 3)

Total bibliographical references were entered into 2 excel tables, with standard information (authors, date, title of article, of journal, volume, pages…) and a few key-words categorized as follows:

Ref. 2 (molecular studies on PRM): Kind of molecular exploration, Objective, Method(s), Target organism (PRM or associated bacteria, mites…)

Ref. 3 (resistances in arthropods): Kind of article (original article, review), Resistance mechanism, Arthropod group, Insect-/Acaricide family/molecule, Characterization tools

Besides, a column was dedicated to identify article with a corresponding pdf file and articles with no pdf file available.

Each excel file were checked for consistence and completion, and the presence/absence of associated pdf. The lacking pdf files should be provided in the future thanks to the different access to libraries among COREMI participants and/or following requests to authors.

On the whole, twenty different articles were listed in the ref. 2 table, and all corresponding pdf files were made available in the associated folder. Thirty-eight different articles were listed in the ref. 3 table, with 17 pdf files available. The 21 remaining pdf files should be asked in the future.

It was discussed about how to standardize the file titles and to make both the reference table and the pdf files available to the COREMI participants, without facing problems with copyright (no total public access, since some of the pdf are not open access).

Summary of available deliverables from collaborative works

Obj. 1: a rough overview of labs with molecular biology facilities and PRM biorepositories available in the COREMI network and suggestions for a future more efficient and exhaustive survey (appendix 1)

Obj. 2: an excel table with 21 literature references (original articles) using molecular tools on PRM + a folder containing the 21 corresponding pdf files (currently available on google drive)

Obj. 3: an excel table with 38 literature references on both reviews and original articles involving genomic data available on arthropods + a folder containing the 17 of the 38 corresponding pdf files (currently available on google drive)

Discussions and perspectives (second part of session 1 and whole session 2)

Discussions on three different topics were initiated based on introductory talks by 4 speakers. These discussions were dedicated to allow participants getting a view of how molecular biology tools may allow different approaches to be developed and different scientific questions to be answered, and to encourage thoughts about perspectives in the COREMI framework. The expected outputs were designing perspectives for 2016 and further and identify possible interactions between present and absent COREMI participants in the future to progress this issue.

Discussion on the genetic bases of resistance

A discussion on the genetic bases of resistances, with an introductory talk by Kathryn Bartley on detoxifying enzymes and another one by Marianna Marangi on target resistances was started.

The two introductory talks allowed people getting a view of some much promising studies currently in process. KB presented important results from a study based on transcriptomics, in vitro expression and bioassays on the different glutathion S-transferases (GST) in PRM and their respective affinity with acaricides. Transcriptomic analyses allowed to identify 11 GST contigs belonging to 4 GST classes (of the 10 known in insects. They were expressed in *E. coli* to investigate their activity and affinity with acaricides via competitive inhibitory assays. Important interaction between recDegGST-1 with phoxim, permethrin, abamectin and nitrobenzyl?? was recorded. It was concluded that targeting GST in the context of conventional control could offer the possibility of improving acaricide efficiency. MM presented a starting project dedicated to identify target resistances against the main synthetic molecules used in layer farms against PRM. The longer term aim of this starting project is to identify non synonymous mutations in cDNA of target proteins sodium channel and acetylcholinesterase associated with resistant phenotypes and to assess their frequency in different farms.

The proposed question to be discussed was: how to encourage and structure molecular studies to better understand (1) resistance mechanisms, (2) resistance epidemiology? However, because time was spent out, the discussion was just started and did not allow reaching clear outputs… Anyway, this

allowed people to be aware about some currently ongoing works on PRM resistance in the COREMI consortium, and resistance issues were dealt with during subsequent discussions.

Discussion on ecosystem approaches

A discussion was initiated on biotic interactions in bird-arthropod system and the interest of molecular tools to better understand how hematophagous arthropods behave in a bird ecosystem. The discussion was introduced by Dieter Heylen, who presented a case study from the wild avifauna. He provided us with a synthesis of a 9-year integrative study of a host-tick-pathogen system in wild passeriform birds. The biology of three close species of *Ixodes* ticks (*I. arboricola*, I. *frontalis, I ricinus*) and their interactions with mainly two tit hosts (*Parus major, Cyanistes caeruleus*) and several *Borrelia* populations (pathogenic genus of bacteria, encompassing the agent of the Lyme disease) was studied using a diversity of methods.

DH highlighted how important it was to consider the different components of biotic interactions to understand how pathogenic bacteria and the tick vectors affect the birds, and illustrated how simple molecular genotyping may contribute to such investigation. Complex interactions between tick community, bird community and pathogen community depend on host prevalence, vector-host contact rate, vector prevalence, vector competence and a diversity of other factors, such as,for instance, the spatial distribution of the different organisms (e.g., due to different foraging niches between 2 tit species (resp. predatory of spiders and caterpillar), the tick community composition and temporal dynamics revealed to be very different (preferred preys found at different heights: spiders (high), caterpillars (low, close to roots)…). DH studied the possible reservoir role of birds in mammalian infections by *Borrelia* using simple molecular markers to characterize two-level structures in the *Borrelia* communities (genospecies, genotypes within genospecies) from ticks collected in bird and mammal environments. DH results revealed that *Borrelia* encompasses different genospecies more or less host specialized. *I. frontalis* and *I. arboricola* did not share any genospecies, while there was some shared genospecies between *I. ricinus* (known to be a vector of *B. burgdhorferi*) and each of the two other species. Mammalian genospecies survived bird host transfer. However, important changes in the *Borrelia* communities and genotypic composition within *B. garinii* were recorded depending on the bird species in host transfer experiments.

The proposed question for discussion was: why and how to encourage and structure ecosystemic approaches to better understand the specific biology of PRM and how control/prevention actions work?

The discussion led to evoke several different perspectives for future studies to be encouraged. It was highlighted how much important it is to investigate the farm building ecosystems to better understand interactions between hens, PRM and associated organisms. Especially, the highly heterogenous farm space implies that a diversity of microhabitat exists, which should be defined. Some thoughts were proposed about how to connect the knowledge on PRM biology and improvement of control. Indeed, as ticks, PRM is a hematophagous arthropod who spends most of their life off host (even more striking in PRM). Consequently, it is crucial to take into account the influence of abiotic conditions and current knowledge about PRM tolerance ranges (temperature, submersion…).

Some questions which could be usefully addressed using molecular tools to contribute to PRM management were listed.

Discussion on the utility of transcriptomics

A more global discussion was initiated on the utility of transcriptomics to better investigate the PRM biology. Harry Wright introduced this part of the session by a talk providing results from a recent study on PRM transcriptome with a focus on adaptations to haematophagous parasitic lifestyle. Transcriptomic data obtained using a 454 Roche platform from a mixed PRM population sampled from a commercial facility were analyzed and compared with three other mite species having different feeding strategies: *Psoroptes ovis* (Astigmata, feeder of sheep exsudates), *Metaseiulus occidentalis* (Dermanyssina as PRM, predatory mite feeding on phytophagous arthropods), *Tetranychus urticae* (Trombidiformes, phytophagous mite). The percent of homologous regions between the annotated PRM transcriptome (using blast2go) and these mites' was stated using tblastx in BLAST. It appeared that the most important homology % was by far with *M. occidentalis* (the most closely related species under test here). Sequences homologous to different pathogenic microorganisms common in hematophagous or hemolymphophagous arthropods were recorded from the PRM transcriptome: Ngewotan and Wallerfield viruses (typical of hematophagous mites), *Bartonella schoenbuchensis* (a bacterium transmitted by blood-sucking arthropods). Other pathogenic microorganisms associated with arthropods were recorded: Israeli acute paralysis virus (a virus involved in colony collapse disorder in honeybees), BrBV (a virus isolated from the aphid *Brevicoryne brassicae*). Some homologues of proteins involved in the innate immunity of arthropods were also reported, as well as of proteins involved in insecticide resistance (though no sequence homologous to the voltage gated sodium channel – the protein target of the commonly used pyrethroid insecticides - was identified to date in the dataset). As for feeding strategy differences between the 4 mites under test, some homologue of leukotriene A4 hydrolase (aminopeptidase) was recorded in carnivorous (PRM, *M. occidentalis, Psoroptes ovis*) rather than herbivorous *T. urticae.*

The proposed question was: What would be interesting to be done using transcriptomics tools to better understand PRM and improve its management?

The Harry's talk was a perfect case in point of a transcriptomic study on PRM. Currently, among participants, studies on PRM transcriptomics are mainly dedicated to identify candidate genes for vaccine development (Harry, Kathryn). Thanks to the use of Genbank and bioinformatics tools, obtained data may be used for different purpose, as demonstrated during the discussions in topic 1 and 3.

Methodological issues were discussed, including advantages/flaws of different NGS methods (Roche 454 vs Illumina), utility of RNA seq to find mutations, importance of coupling proteomics to transcriptomics to identify the most important proteins, some warnings about the need for good quality RNA (need to pool individuals…). Some suggestions were proposed for future studies aimed at improve knowledge on enzymes involved in the blood digestion, mutation rate associated with acaricide resistances.

Suggestion for future research

Summary of the assessment and brainstorm session

- Crucial = keep in mind biology when using molecular tools: combine bioassays to molecular explorations, check proteomics when doing transcriptomics…
- /!\ Pb of amount and quality of DNA/RNA…
- Lit. ref. database: choose a standard format
    - Name – et al – date 10 first words of title (suggestion by EP)
    - Jag will ask the administrator of the COREMI website

    - Collaborative work (either on google drive, as now, or on the COREMI website): ask people to update/enrich the database, provide lacking pdf files if possible …
- Neutral genotyping to improve prevention and control: salmonella genotypes and differences in the vectorial role of PRM, PRM spread and resistance dispersal…
- Barcoding methods (individual barcoding, environmental DNA) may allow progress in the ecosystemic investigation of PRM habitats
- Coupled transcriptomics + proteomics studies susceptible to progress in the
    - Development of vaccines (in process, Moredun, UK)
    - Understanding of adaptation to hematophagy (enzyme activity in feces…) (a study to be started?)
    - Progress the knowledge of resistances against acaricides (maybe connexion to be implemented with T. Van Leeuwen – not available invited people, but did contribute to the resistance literature database and told that a review on resistances in mite will be published within the next few years)

Current works and possible interactions among participants?

- People involved in currently ongoing/recent studies on PRM:
    - Kathryn and Harry on vaccine development
    - Kathryn on GST and metabolic resistances
    - M. Marino on Salmonella-PRM-hen relations,
    - EP, LR and MEA starting works on predatory mite communities
- Possible new collaborations among participants of the workshop
    - EP / LR-MEA and DH and Erik Matthysen (Antwerpen, B) on wild bird systems
    - Harry / Marianna on insecticide target proteins
- Possible new collaborations with other people
    - T. Van Leeuwen on resistances (NL – took part to collaborative work)
    - R. Finn on resistances (UK)

WG4

The activities of the WG4 during the workshop in Montpellier were mainly focused on two aspects: the vectorial role of the poultry red mite (PRM) *Dermanyssus gallinae*, and the current knowledge about the epidemiology of the parasite.

Vectorial role of PRM

The first topic was the central theme of the morning session held on Thursday. In his introduction, prof. Antonio Camarda, stressed the wide diffusion of PRM and its effects on the poultry production system. Among the latters, there is the possible role played by the parasite in the diffusion of infectious disease. The discussion was centered about this query: past literature described the association between *D. gallinae* and a number of pathogens, but very scarce information is provided about its actual role in the vectorial transmission of the diseases. Therefore, how would be possible to define this?

Considering the growing difficulties in the setting up of experimental infections, also due to ethical and administrative reasons, the group agreed that the network of researchers, experts and operators building up by the COREMI COST action may be very helpful, as it will allow to exchange knowledge, research strategies, and, in particular to have the possibility to perform investigations in poultry flocks in which there were concurrently the PRM infestation and a pathogen infection.

In the light of this field cases proposed from Antonio Camarda have been discussed and explored.

In the meantime, the group also agreed to draft a review paper, in which collect the present-day strategies and methods which have been used, or which be potentially used, to assess the vectorial role of *D. gallinae*.

Epidemiology of the parasite

The prevalence and diffusion of the PRM was the main theme of the second session. Dr. Stewart Plaistow, from the Institute of Biology of the University of Liverpool, introduced the topic with a short lecture about the population dynamics of mites, exploring the involved factors, analyzing how populations respond to environmental changes, and the dispersal mechanisms of mites.

The following discussion was aimed to the application of the theoretical aspects to the daily experience with *D. gallinae* in poultry farm. The attendants discussed together how PRM population might be influenced by the availability of meal, the temperature (with particular attention to the cold environment), and about the variables which might affect the population size or movement of mites among farms.

Finally, in order to put the basis for a shared method of data collection about prevalence and diffusion of *D. gallinae*, a questionnaire was defined, to be administered to the farmers. The participants agreed that the questionnaire should collect not only data about the presence of the mite in the flocks, but also the possible factors which might have enable the introduction and persistence of the parasite in the farm. The group also acknowledged that the involvement of the national associations of producers may be very useful to facilitate the administration of the questionnaires, and the collection of reliable data.

Sum-up of works and links between WG

Overall, work and discussions were very fruitful and raised lots of questions. Here is a short overview of identified needs and planned actions:

Recorded/highlighted

- WG1: need for investigations on resistance issues and
- WG2: useful insights about improvement/standardization of monitoring tools (Frequency, duration and methodology to be used)
- WG3: need for molecular training with systematic consideration of biological assays
- WG4: importance of exploring PRM vectorial role / Salmonella and their respective prevalence

Planned common work:

- WG1-3-4: A questionnaire to be distributed in Europe, involving ECIs if possible for data analysing. Questionnaires will need to organised in each country by a co-ordinator selected within MC or WG members and translated in national languages
- WG1: A paper to be finalised on control methods should be prepared in 2016.
- WG3: Creation of a biobank of samples (with interest of WG1)
- All four WG: make possible the growing of literature databases available to the COREMI network (see below). Currently 2 folders are available from google drive, and should be completed by all COREMI participants and if possible transferred to the COREMI website:
    - https://drive.google.com/folderview?id=0B5TF_BfwjpU5dW4wUmFCU2IxMDA&usp=sharing: an excel table with 21 literature references (original articles) using molecular tools on PRM + a folder containing the 21 corresponding pdf files (currently available on google drive)
    - https://drive.google.com/folderview?id=0B5TF_BfwjpU5bVAxLUVUT2hGNWs&usp=sharing: an excel table with 38 literature references on both reviews and original articles involving genomic data available on arthropods + a folder containing the 17 of the 38 corresponding pdf files (currently available on google drive)

Evaluation by participants

At the end of the workshop, participants were asked to write comments on coloured post-it and to stick them on a wall in the plenary room.

Overall, comments on the organisation and contents highlighted the richness of discussions and knowledge exchanges. Both advantages and disadvantages were found to be associated with the particular organisation with parallel working sessions: this allowed discussing about lots of diverse things (more than if all in plenary sessions), but was frustrating in some way since people may be interested in two subjects discussed at the same moment. This was a relatively successful "experimental" organisation. Some suggestions were made which should be useful in the framework of some such future events (see below): e.g., a plenary working session (not only plenary sessions for brainstorm or summary) and the reduction of the brainstorm sessions time.

Please find below all comments:

Green: suggestions for new discussions

- What would be the objectives for a future EU-financed project?
- Optimal system for keeping/rearing PRM

- Introducing the most common and most successful applied treatments in different countries
- A little book with all the topics discussed
- Maybe will be useful to discuss and to update the information regarding the new products released on the market (acaricides)
- Central resource of PRM publications on website
- Ask a poultry practitioner to give the practitioner/field perspective
- Invite a human parasitologist to talk about PRM reactions in humans
- "COREMIBOOK" platform for red mite society
- Choose one test for testing efficacy acaricides
- The subjects addressed to specific conditions for breeding of birds in different countries
- I think the one health workshop is very interesting to map the possible disadvantages/diseases/ €€ of PRM infections
- About Salmonella I think there are a lot of misunderstandings. Maybe we need some experts on Salmonella

Blue: what was good about the workshop

- WG .... (initiatifs?) better prepared by all participants when possible
- ...(diveis) group of researchers/sector/ industry from different points/parts of me
- I shared information about all working groups
- Efficient way to get up to speed in correct state of understanding Thanks, great job
- Meeting people with some interest exchange ideas
- Best...efficiently organised. Thank you
- Working group workshop (WG2) was very well prepared
- Good speech. Excellent connection of ideas
- 1. Organisation : venue, city, food, wine. 2. Work in smaller groups; possibility to hear other group discussions
- Interactions of participants in various fields and areas related to PRM
- Good organisation. Good WG leaders coordination. Nice discussions
- Good alternance and possibility of collaboration between working groups
- WG working separately: best thing and worst thing! Best because in few people... and topics maybe fully addressed. Worts because sometimes participants miss interesting results. Maybe some additional plenary sessions would solve the problem (which is overlap of session so unable to attend all sessions), maintaining all benefits of the WGs parallel works

Red: suggestions for improvement

- Provide delegate list with a sketch of expertise before the start. Maybe online. Thank you.
- A little workshop with all the members of the group
- Wider involvement of young investigators
- Participation of other groups in a WG session: OK!
- Come on, it is perfect

- My suggestion for a next workshop is to reduce the brainstorm sessions time to reduce some repetitions. Another suggestion is to better connect two different groups about the same issue, prevalence questionnaires for instance. Thank you of all and cheers.
- Maintain at least 1 session with everybody together

Appendix 1: results from the 1st tentative survey on molecular biology facilities and biorepositories that are available to the COREMI consortium

Contact info

We asked the participant to fulfil their name, their country and their e-mail addresses. It would be interesting to ask also for the affiliation of the person in order to see 1) the intra-country differences and 2) in which kind of structure the "respondent" work in.

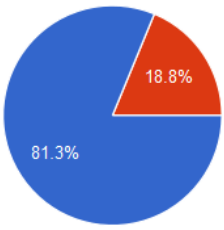
Origin of the participants :

Country	**Number of response**
Belgium	2
Croatia	1
Denmark	1
Israel	2
Italy	4
Norway	1
Portugal	1
UK	3
Total	**15**

Information on Lab

We ask for the participants to indicate if they knew any lab having skills and/or tools in molecular analyses of any arthropod group. Most responders answered they knew at least one.

Do you know any lab having skills and/or tools in molecular analyses of any arthropod group?

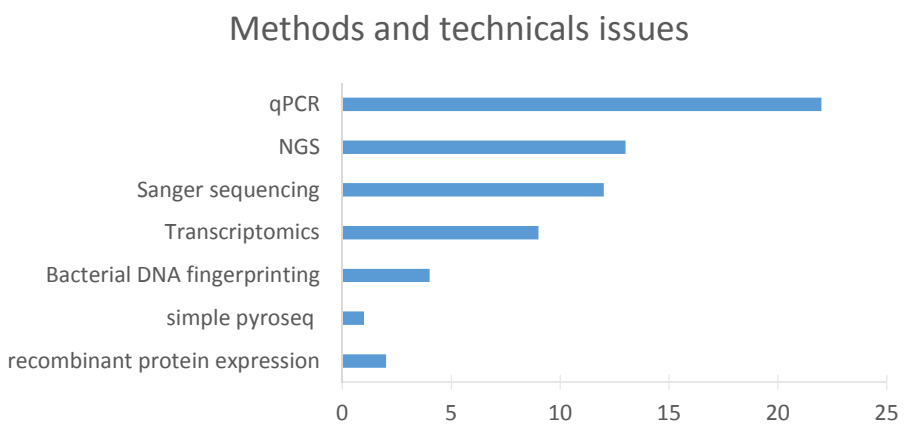


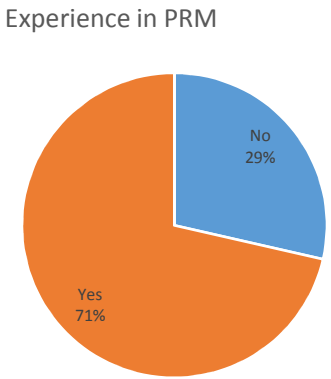
Yes	**13**	81.3%
No	**3**	18.8%

In view to improve our questionnaire, we think it will be best to ask directly the contact information of the other lab (one person contact, type of institute and country), in order to avoid redundancy in answers. Indeed, this is possible that the participant knows the same laboratories.

Moreover, we only let people inform 3 labs and maybe they know more.

Concerning the skills, the most common techniques (qPCR, NGS, Sanger sequencing) are much represented in the responses (see page 2).





Methods and technicals issues depending on the origin of the participant

Countries	qPCR	NGS	Sanger sequencing	Transcriptomics	Bacterial DNA fingerprinting	recombinant protein expression	simple pyroseq	Total
Belgium	3		3	2				**8**
Croatia	2		1					**3**
Israel	5	3	3	2	3			**16**
Italy	7	6	4					**17**
Norway	1		1		1		1	**4**
Portugal	2	2		2				**6**
UK	2	2		3		2		**9**
Total	**22**	**13**	**12**	**9**	**4**	**2**	**1**	**63**

In the future questionnaire, maybe we can focus on “rare” techniques or very particular/technical ones because the basic ones are very commonly accessible.

Living biological material

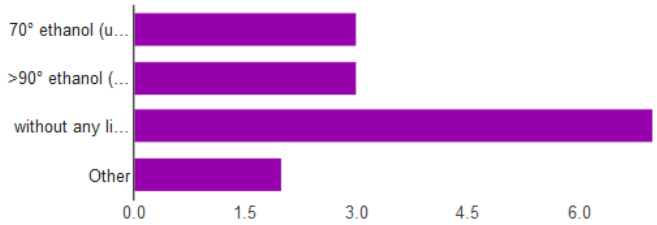
Concerning the availability of living mite culture, there was a misunderstanding on the meaning of “mite culture” and the responses inclued a lot of field population (in farms) and not laboratory culture of mite as expected.

There is almost 1 laboratory who maintain mite for routine analysis. It is an academic lab which possesses 3 populations of sensitive PRM (against insecticides).

Biorepositories

Apparently there is a lot of different samples of dead mites. They are mainly stored without any liquid and preserved at -20°C, which is the most suitable for most of molecular analyses.

Preservation medium



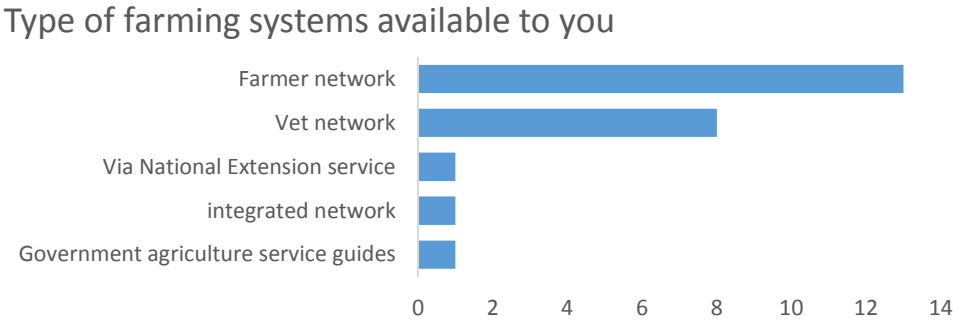
70° ethanol (undenatured alcohol)	3	27.3%
>90° ethanol (undenatured alcohol)	3	27.3%
without any liquid	7	63.6%
Other	2	18.2%

Preservation temperature

frozen at -20°C	9	64.3%
frozen at -80°C	5	35.7%
room temperature	3	21.4%

Sampling network

The sampling network of the participant is mostly composed of farmers or vets. We don’t have any info about how it is easy/allowed to enter farms and get samples. In some countries, this could be difficult even if you are in contact with professional’s poultry networks.



In the participant network, the farming systems most represented are free range and cages but there is also others system available. Most of these farming systems are managed in a conventional way.

Other aspects should be taken into account to characterise PRM management such as direct or indirect PRM signs, type of treatment, prevention…